1091-Pos Board B42
Multimerization of Solution-State Proteins by Anionic Porphyrins
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Protein-protein interactions and formation of multi-subunit protein complexes remain among the most challenging areas of modern Biophysics. Recently, surface binding and interactions of anionic porphyins with cationic proteins gained a lot of attention as relevant models for protein surface molecular recognition and photoinitiated electron transfer. However, interpretation of data in nearly all reports explicitly or implicitly assumed interaction of porphyrin with monodisperse proteins in solutions. Here, using small-angle X-ray scattering with solution phase samples, we demonstrate that horse heart cytochrome (cyt) c, triheme cytochrome c7 PpcA from Geobacter sulfurreducens, and hen egg lysozyme multimerize in the presence of several water-soluble porphyrins. Multimerization of cyt c induced by tetrakis(4-sulfonatophenyl)porphyrin showed a pH dependence with a stronger apparent binding affinity under alkaline conditions and was weakened in the presence of a high salt concentration. Ferric-cyt c formed complexes larger than those formed by ferro-cyt c. Free base TPPS and FeTPPS facilitated formation of complexes larger than those of ZnTPPS. A number of carboxylated porphyrins induced multimer formation as well. No increase in protein aggregation state for cationic proteins was observed in the presence of cationic porphyrins or sulfonated anthraquinone. All-atom molecular dynamics simulations of cyt c and PpcA with free base TPPS corroborated X-ray scattering results and revealed a mechanism by which the tetrasubstituted charged porphyrins serve as bridging ligands nucleating multimerization of the complementarily charged protein. The final aggregation products suggest that multimerization involves a combination of electrostatic and hydrophobic interactions. The results demonstrate an overlooked complexity in the design of multifunctional ligands for protein surface recognition.

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Characterization of the Photophysical, Thermodynamic and Structural Properties of the Terbium(III)-KChIP3 Complex
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KChIP3 (K^+ channel interacting protein 3) is a calcium binding protein and active modulator of Kv4 channels in neuronal cells as well as a novel Ca^{2+} regulated transcriptional modulator. KChIP3(calsenilin) may also be involved in Alzheimer's disease through prevention of presenilin-2 fragmentation. Many of the KChIP3 interactions with its binding partners (Kv4, calmodulin, DNA, and drugs) have been shown to be dependent on calcium. Therefore, understanding the structural changes induced by Ca^{2+} is of utmost relevance to elucidating the mechanism of calcium signal transduction. Here, we show that the fluorescence emission and excitation spectra of the calcium luminescent analog Tb3+ is enhanced upon binding to the EF-hand of KChIP3, likely due to a mechanism of energy transfer between Phe/Trp and Tb3+. We also observe that unlike Tb3+ bound calmodulin, the luminescence lifetime of terbium bound to KChIP3 decays as a complex multiexponential (τaverage ~ 1.8 ms) and is sensitive to the protein structure and drug (NS5806) binding. Using isothermal calorimetry we have determined that Tb3+ binds at least to four binding sites (Kd ~ 2.2 μM) and is able to displace bound Ca^{2+} through an entropically driven mechanism (ΔH ~ 3 kcal/mol). Secondary structural analysis of KChIP3 using far-UV CD spectroscopy shows that binding of Tb3+ induces the formation of an intermediate structure with less alpha helical content than that induced by Ca^{2+}. However, using the hydrophobic probe 1,8-ANS we show that the structural changes induced by Tb3+ are large enough to expose a hydrophobic surface on KChIP3 identical to Ca^{2+} bound protein. Similarly to Ca^{2+}, terbium binding also induces the dimerization of KChIP3. Overall, these results support the use of Tb3+ as an alternative fluorescent label in the study of Ca^{2+} induced structural changes in KChIP3.

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Simulations of Cosolute Effects on Protein's Stability
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Cosolutes are small miscible organic molecules that affect the solubility of proteins. Many different kinds of cosolutes have been observed to affect the conformational equilibrium of proteins in solution. Of special consideration are cosolutes that are able to denature (urea), stabilize (TMAO), or prevent aggregation (arginine). We take under consideration a special case of cosolutes termed osmolytes that have a sizable effect on the osmotic pressure of the solution. In particular, we are interested in how simple amino acids (Arg, Pro, Gly) increase the stability and solubility of proteins. We use molecular dynamics simulations to study the interactions of these cosolutes with proteins. The system under study is the mini protein trp cage, simulated in a variety of solutions consisting of different amino acids as cosolutes, in ensembles that are representative of the folded and the unfolded states. We calculate the osmotic pressure to measure the cosolute-cosolute interactions, and the preferential interaction coefficients to further assess the protein-cosolute interactions. These calculations provide information about the interactions of various amino acids with proteins. The calibration and validation with experimental data provide a way of refining force field parameters for modeling proteins in solution.

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Interactions of Amide Solutes with Biopolymer Functional Groups and Hofmeister Salts
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Urea and other amides exert large destabilizing effects on noncovalent biopolymer self-assembly and binding. Salt anions and cations from the Hofmeister series exert characteristic stabilizing or destabilizing effects on these processes. Effects of these small solutes and salt ions arise from their favorable or unfavorable interactions with functional groups on the protein or nucleic acid surface exposed. To quantify these interactions, we determine the thermodynamics of interactions of amide solutes with model compounds displaying the functional groups of interest (other amides, aromatic hydrocarbons, nucleobases) and with Hofmeister salts using osmometry or solubility assays. Multivariable linear regression of these and literature data reveals that these solute-functional group interactions are additive and generally independent of context, and yields interaction potentials (α-values) quantifying interactions of each solute with amide, hydrocarbon and nucleobase groups. These results allow us to predict and/or interpret effects of these solutes on protein and nucleic acid processes, and to use these solutes as probes of large conformation changes in transition states and intermediates. Analysis of thermodynamic data for interactions of a series of alkylated ureas with different surface types reveals that favorable interactions with sp^2 and sp^3 C and N and unfavorable interactions with sp^2 O increase with increasing hydrocarbon surface area of the alkylated urea. Interactions of these alkylated amides with a series of Na^+ and K^+ salts of Hofmeister anions reveal that interactions of the salts with hydrocarbon C and amide N, but not amide O, follow the Hofmeister order ($KSCN > KCl > KF$; $NaClO_4 > NaCl > Na_2SO_4$). Additional analysis of these data should provide information regarding free energies of interaction of C, N and O groups in folding, binding and other noncovalent self-assembly processes of proteins.
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Identification of Inhibitors against P.Falciparum GAP50 and Human Complement Factor H Interaction in the Mosquito
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Malaria is an infectious disease caused by obligate intracellular parasites of the genus *Plasmodium*. Severe malaria cases result in more than half a million deaths every year (World Malaria Report, 2013). Mosquitoes transmit the parasite from human to human during their blood meals. With the blood meal, mosquitoes ingest female and male gametocytes present in the blood of a *Plasmodium* infected human. Gametocytes develop into gametes (Aly *et al*, 2009) that undergo fertilization inside the mosquito's gut and later result in the formation of infectious sporozoites that are transmitted during a bite. This uptake, maturation and fertilization, represents a bottleneck in the parasite's life cycle, and a prime target for transmission blocking. In the mosquito's gut, macrogametes (female gametes) bind the human complement control regulator protein, hFactor H, via *Pf*GAP50 to avoid complement-mediated lysis (Simon *et al,* 2013). A potential transmission-blocking small molecule inhibitor would disrupt the *Pf*GAP50-hFactor H interaction, exposing the parasite to the human alternate pathway of complement for destruction.
The goal of this project is to characterize the *Pf*GAP50-hFactor H interaction *in vitro*, and to screen the Medicines for Malaria Venture Malaria Box library for small molecule protein-protein interaction (PPI) inhibitors using a surface